A Rough Colony Morphology of *Mycobacterium abscessus* Is Associated With Cavitary Pulmonary Disease and Poor Clinical Outcome

Wilhelm Hedin,1 Gabrielle Fröberg,1,2 Kalle Fredman,3 Erja Chryssanthou,1 Ingrid Selmyrd,4 Anna Gillman,5 Letizia Orsini,6 Michael Runold,7 Bodil Jönsson,8 Thomas Schön,9,10 and Lina Davies Forsman1,10

1Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden; 2Department of Medicine, Division of Infectious Diseases, Karolinska Institutet, Solna, Sweden; 3Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden; 4Department of Infectious Diseases, Västmanland Hospital, Västerås, Sweden; 5Department of Medical Sciences, Section for Infectious Diseases, Uppsala University, Uppsala, Sweden; 6Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Solna, Sweden; 7Department of Medicine, Division of Respiratory Medicine and Allergology, Karolinska Institutet, Solna, Sweden; 8Clinical Microbiology, Sahlgrenska University Hospital, Gothenburg, Sweden; 9Department of Infectious Diseases, Region Östergötland and Kalmar County Hospital, Sweden; and 10Department of Infectious Diseases, Karolinska University Hospital, Solna, Sweden

Background. The *Mycobacterium abscessus* complex (MABC) is a difficult to treat mycobacterium with two distinct morphologies: smooth and rough. As the clinical implications are unclear, we explored the morphology of MABC in relation to disease and outcome.

Methods. We performed a retrospective multicenter cohort study including patients with confirmed MABC in Sweden, 2009–2020, with treatment outcome as the primary outcome. MABC colony morphology was determined by light microscopy on Middlebrook 7H10 agar plates.

Results. Of the 71 MABC isolates, a defined morphology could be determined for 63 isolates, of which 40 were smooth (56%) and 23 were rough (32%). Immunosuppression, pulmonary disease, and cavitary lesion on chest radiographs were significantly associated with a rough isolate morphology. Participants with smooth isolates had more favorable treatment outcomes (12/14, 86%) compared to those with rough isolates (3/10, 30%). In an age-adjusted logistic regression, rough morphology of MABC was associated to lower odds of clinical cure compared to smooth morphology (adjusted odds ratio, 0.12; \( P = 0.049 \)).

Conclusions. Study participants with rough MABC colony morphology of isolates had a worse clinical outcome compared to those with smooth isolates. The biological mechanisms should be further characterized and colony morphology of MABC taken into account during clinical management.

Keywords. nontuberculous mycobacteria; colony morphology; *Mycobacterium abscessus* complex; subspecies; treatment outcome.

In many high-income settings, an increase in the proportion of mycobacterial disease caused by nontuberculous mycobacteria (NTM), as opposed to *Mycobacterium tuberculosis*, has been observed. The most common manifestation of NTM infection is pulmonary disease, but nearly all organs can be affected [1]. *Mycobacterium abscessus* complex (MABC) is one of the most clinically relevant, rapidly growing mycobacteria, consisting of 3 subspecies: subspecies (subsp) *abscessus*, subsp *Massiliense*, and subsp *bolletii* [1, 2]. Infections with MABC are notoriously difficult to treat, due to intrinsic drug resistance in combination with potential adaptive and acquired resistance [2, 3].

Diagnosis and treatment of MABC pulmonary disease is complex and based on limited scientific evidence. A combination of clinical, radiographic, and microbiologic criteria has been proposed to aid clinicians in the diagnosis of NTM pulmonary disease [4]. The treatment includes an initial phase of combined oral and parenteral therapy, including at least 3 active drugs, such as a macrolide, imipenem, tigecycline, or amikacin. The continuation phase is recommended to include at least 2–3 active oral drugs. Macrolides are critical for a successful treatment outcome of MABC disease [5]. Inducible macrolide resistance is conveyed by a C28T alteration, that is some methylase (erm)41 gene, found in subsp *bolletii* and the majority of subsp *abscessus* [6, 7]. Subsp *massiliense* harbors a nonfunctional *erm* and does not exhibit inducible macrolide resistance [7]. The optimal duration of therapy for pulmonary disease is not known but is often around 12–18 months [3, 4]. Successful treatment outcomes of pulmonary MABC disease ranged from 41% to 46% for all subspecies combined in 2 meta-
analyses [5, 8]. Treatment success rates across subspecies is highly variable and has been reported as 33% for subsp absces-
sus and 57% for subsp massiliense [5]. Treatment success rates
are further reduced in cases of cystic fibrosis where NTM infec-
tions are often of a chronic nature [9].

MABC has 2 distinct macroscopic morphologies, termed
smooth and rough based on the appearance of cultured colo-
ries (Figure 1) [10–12]. The smooth colonies are shiny and
smooth, resembling the reference strain M. abscessus subsp
abscessus American Type Culture Collection (ATCC) 19977. Rough
morphology is characterized by larger and markedly
rugged, waxier colonies [13]. The smooth morphotype express-
es high levels of glycopeptidolipids in the cell wall, giving it the
ability to form biofilms in vitro [14]. In comparison, the rough
morphotype has reduced amounts of glycopeptidolipids in the
cell wall and exhibits cording to a larger extent, a property
associated with mycobacterial virulence [1, 14].

Colony morphology of MABC is currently rarely routinely
reported and although limited microbiological data are avail-
able, the clinical importance of this phenomenon remains to
be investigated. Therefore, we performed a retrospective cohort
study with the aim of studying the association between rough
and smooth colony morphology in relation to the clinical char-
acteristics and outcomes in patients with MABC infections,
without concomitant cystic fibrosis.

METHODS

Study Population and Setting
We performed a retrospective observational cohort study, includ-
ing consecutive patients with a culture-verified MABC isolate in
Stockholm, as well as MABC isolates sent to Karolinska
University Hospital, Stockholm for drug susceptibility testing
from the cities of Linköping, Kalmar, Västerås, and Uppsala be-
tween 2009 and 2020. Patients with concomitant active pulmo-
nary tuberculosis were excluded as well as patients with cystic
fibrosis, due to the more chronic nature of their MABC infections.

Identification of Isolates and Determination of Colony Morphology
Stored MABC isolates collected from study participants were
thawed and recultured aerobically on Löwenstein-Jensen medi-
un at 30°C, according to accredited clinical routine based on
Clinical and Laboratory Standards Institute (CLSI) standards.
The isolate submitted for culture closest in time to the date
of treatment initiation, or the most recent isolate if the patient
was never treated, was included in the study. The aim was to
culture a sample before treatment initiation, but isolates col-
clected afterwards were exceptionally accepted. Only 1 isolate
per patient was included.

Fluorescent microscopy for detection of mycobacteria and
cording had been done upon diagnosis according to routine
procedures at the laboratory. MABC isolates were recultured
from Löwenstein-Jensen medium onto Middlebrook 7H10
with oleic albumin dextrose catalase agar plates (Merck) and in-
cubated at 30°C for 3–5 days. Identification of MABC subspecies
(subsp abscessus, massiliense, and bolletii, respectively) and the
determination of inducible (erm) or chromosomal (rrl) macro-
lide resistance, as well as aminoglycoside resistance (rps), was
performed by a line probe assay (GenoType NTM-DR; Hain
LifeSciences) [15] and performed after reculturing of the isolates
where this information was missing at the time of diagnosis.

The colony morphology was determined by light microscopy
by 1 laboratory technician and 1 medical doctor- G. F. at the
specialized mycobacteriology laboratory (Karolinska
Hospital), who were both blinded to all clinical data including
outcome to avoid bias. Morphology was categorized as smooth
or rough as previously described and photographs (Figure 1)
were used as a visual aid for categorization [10–12]. Colonies
that contained a mixture of smooth and rough colonies were
categorized as mixed morphology [10].

Data Collection and Definitions
The medical records were reviewed by 2 residents and 4 consul-
tants in infectious diseases or pulmonology using a predesigned
electronic form in REDCap. A 10% overlap with double entry
of data was performed for quality control, where minimal dis-
crepancies were seen and discussed with author L. D. F.

Data were collected regarding demographics (gender, age),
clinical characteristics (pregnancy, comorbidities, previous
tuberculosis/NTM infection, steroid treatment, immunosuppres-
sion, malignancy, human immunodeficiency virus [HIV]), clini-
cal presentation (symptoms, site of infection, radiological
pattern, baseline erythrocyte sedimentation rate, hemoglobin,
and albumin), investigations (bronchoscopy, sputum cultures),
treatment (drug, dose, duration, hospitalization), and treatment
outcome (clinical cure, treatment failure, death all cause, death
due to NTM, and recurrence). Disseminated disease was defined
as infection located at least in 2 separate organs. MABC disease
was defined as the fulfilment of American Thoracic Society/
Infectious Diseases Society of America (ATS/IDSA) criteria [4]
except for cavitary disease, as defined by the responsible clinician. The
clinical and microbiologic criteria for diagnosis of nontuberculous
mycobacterial pulmonary disease consists of (1) pulmonary or
systemic symptoms, (2) typical radiological abnormalities, and
(3) at least 2 positive sputum cultures or 1 positive culture from
bronchial lavage [4]. The combined category “infection/coloniza-
tion” was defined as a microbiological finding not fulfilling ATS/
IDSA criteria of NTM disease with or without symptoms.

Patients were followed uniformly from treatment completion
up to 1 year after. If no treatment was given, the patients were
followed 1 year after diagnosis. The Nontuberculous
Mycobacteria-Network European Trials Group (NTM-NET)
consensus statement was used for treatment outcome definitions
[16]. Microbiological cure was defined as the finding of multiple
consecutive negative but no positive cultures with the causative species from respiratory samples after culture conversion and until the end of antimycobacterial treatment. Clinical cure was defined as patient-reported and/or objective improvement of symptoms during antimycobacterial treatment, sustained until at least the end of treatment, but no cultures available to prove culture conversion or microbiological cure. Because sputum samples were not uniformly collected for the study participants, clinical cure was used as the main outcome of the study. Treatment failure was defined as the reemergence of multiple positive cultures or persistence of positive cultures with the causative species from respiratory samples after ≥12 months of antimycobacterial treatment, while the patient was still on treatment. Death all cause was defined as any death occurring during the disease episode or treatment. Deaths suspected to be caused by the NTM infection [16] were discussed together with the author L. D. F. for uniform assessment. Recurrence was defined as the reemergence of at least 2 positive cultures with the causative species from respiratory samples after cessation of antimycobacterial treatment. The study period ended 31 May 2021 and no outcomes after that date were registered.

**Ethical Considerations**

The study was approved by the Swedish Ethical Review Authority (Dnr 2020-00108).

**Statistical Analysis**

Descriptive variables were summarized with median, interquartile ranges, and absolute and relative frequencies. Comparison of groups was performed with Fisher exact test for categorial data and the Wilcoxon rank-sum test for continuous data. A logistic regression was performed to study the association between morphology (rough/smooth) and treatment outcome, with an adjusted analysis for the identified confounder age. Previously reported potential confounders [3, 8, 17, 18] were explored using univariate analysis and directed acyclic graphs, where age was identified as the main potential confounder. Stata version 16.1 (StataCorp) was used for all statistical analyses. The data for pharmacological treatment were extracted using R version 4.2.0 (2022-04-22 ucrt).

**RESULTS**

**Study Participants**

A total of 71 patients were included and clinical characteristics at baseline are shown in Table 1. A defined colony morphology of smooth or rough could be determined in 63 of the 71 MABC isolates, of which 23 (32%) isolates had a rough morphology and 40 (56%) smooth, without association to subspecies. Four isolates had a mixed morphology type and 4 isolates had been lost (n = 3) or contaminated (n = 1). Previous pulmonary disease, such as bronchiectasis or chronic obstructive pulmonary disease (COPD), was observed in 59% (42/71) of study participants, whereas a positive smear microscopy was seen in 13% (9/71).

**Colony Morphology of MABC: Smooth and Rough**

The morphological appearance of smooth, rough, and mixed is visualized in Figure 1. All the 23 isolates with a rough morphology were isolated from pulmonary samples, whereas smooth morphology was observed from pulmonary samples in 33/40 (83%) of the cases. There was no significant difference in the *erm*(41) 28T genotype between smooth and rough morphology. Cordination was seen more often in isolates with a rough colony morphology than smooth (64% vs 3%, *P* < .001). Immunosuppression, pulmonary origin of sample, and cavitary...
findings on chest radiograph were significantly associated with a rough colony morphology (Table 1). All the participants with a rough isolate presented with a cough, compared to 55% (22/40) with a smooth isolate (P < .001).

**Treatment Outcome**

In total, 18 out of the 27 treated patients were cured (Figure 2). In 3 patients, treatment was started prior to the fulfilment of all NTM diagnostic criteria, due to disease severity and a clear clinical picture of an NTM infection. Of the isolates from treated patients, morphology could be determined for 24 isolates. Following a univariate analysis, the only significant potential confounder that differed between treated participants with rough and smooth morphology was age (n = 24, 73 years vs 58 years, P = .035). There was no difference in the presence of inducible macrolide resistance between smooth and rough isolates (6/14 vs 7/10 respectively, P = .24). Recurrence of infection was seen for 1 of the treated patients.

**Rough Colony Morphology Associated With Worse Treatment Outcome**

A rough morphology was significantly associated with worse outcome, as clinical cure was seen in 86% (12/14) of infections.
with smooth isolates versus 30% (3/10) for participants with rough isolates (odds ratio [OR], 0.07; 95% confidence interval [CI], 0.009–0.54; \( P = 0.01 \)). We performed an age-adjusted logistic regression where the association remained significant (aOR, 0.12; 95% CI, 0.014–0.99; \( P = 0.049 \)). Other potential confounders that were explored included, for example, sex, immunosuppression, bronchiectasis, MABC subspecies, and \( \text{erm} \, 28T \) positivity without changing the significance of the association (data not shown). The association of rough morphology and lower odds of clinical cure was still significant in a subanalysis of participants with pulmonary MABC disease receiving treatment (\( n = 20 \); OR, 0.12; 95% CI, 0.014–0.99; \( P = 0.049 \)). The median time to death of these 5 patients was 109 days after treatment initiation, compared to a median treatment duration of 289 days for all study participants (Table 2). The median number of concomitant drugs was 2 and there were periods of monotherapy for 14 patients, for a median period of 64 days. As shown in Table 2, the treatment regimens for the study participants were similar regardless of colony morphology of the isolate.

Characterization of Study Participants With Infection or Colonization With MABC
In the study population, a total of 31 patients (44%) were categorized as having an infection/colonization rather than MABC disease, based on published criteria including absence of radiological findings, repeated culture findings, or clinical symptoms suggestive of MABC disease [4]. There was no significant association

**Figure 2.** Flow chart of study participants regarding treatment and treatment outcome. * Missing outcome since treatment was ongoing by the end of the study period 31 May 2021.
between subspecies or colony morphology and infection/colonization (Supplementary Table 1 and Table 1). Patients without MABC disease were younger, predominantly male, less often immunosuppressed, had a higher body mass index, and a lower proportion of bronchiectasis and cavitary lesions on radiograph, and none were treated. None of the study participants with an infection/colonization were sputum smear positive at baseline compared to 23% patients with MABC disease (P = .004). Regarding symptoms at baseline, participants with MABC infection/colonization presented less often with fever and weight loss, compared to those with disease (Supplementary Table 1). No one in the infection/colonization group died, compared to 12 out of 40 participants in the disease group.

**DISCUSSION**

In summary, we found that a rough MABC morphology was associated with a poor clinical outcome, with less chance of clinical cure. Furthermore, a rough morphology of MABC was only isolated from pulmonary samples and was associated with immunosuppression as well as signs of more extensive disease burden by cavitary lesions on chest radiograph and a higher frequency of cough in the study participants.

The largest study to date trying to address the question of MABC morphology and clinical outcome is a recent Chinese study by Li et al [19]. This study included 182 patients, of which 65% were infected by a rough MABC isolate. They reported a greater decline in pulmonary function (forced vital capacity), as well as more severe symptoms and more frequent exacerbations in patients with rough MABC isolates, although the final treatment outcome was not reported. However, the baseline characteristics of the patients in the study infected by rough MABC isolates were significantly worse than for smooth isolates and no adjustment for confounders such as age or subspecies was performed. The number of patients included with cystic fibrosis was not reported. Almost half the patients in the study had previously been treated for pulmonary tuberculosis (57% in rough group and 48% in smooth), compared to only 5 (7%) patients in our study.

In a study from South Korea with 62 patients treated for pulmonary MABC disease, all of whom were infected with subspp abscessus, patients with a stable negative sputum conversion more frequently exhibited an isolate with a smooth colony morphology (9/20, 45% vs 2/24, 8%; P = .02) in a crude analysis without adjustment for confounders [17].

Potential confounders, such as variables known to affect treatment outcome, are important to consider during analysis of colony morphology versus outcome. Established risk factors for poor treatment outcomes for MABC infection includes subsp abscessus (most likely due to inducible macrolide resistance [5]), drug regimens without macrolides [5, 17, 18], imipenem, or amikacin [5], positive sputum smear microscopy on diagnosis [17], cavitary lesions on radiograph, previous NTM lung disease, and older age [18]. We explored potential confounders in our analysis, of which age was deemed the most important and adjusted analysis was performed.

There are several theoretical explanations supporting the increased severity of rough MABC infections. The rough variant to a larger extent exhibits cording, a known virulence factor of *M. tuberculosis* [20]. The coiled, multibacterial aggregates lead to inhibition of phagocytosis by macrophages and thereby evade the immune system [21]. Indeed, cording was seen for the majority of the rough isolates in our study. A recent study with a zebrafish model, showed an accelerated granuloma formation of rough compared to smooth MABC isolates [22]. Moreover, in the aforementioned Chinese study by Li et al., the serum levels of inflammatory factors such as C-reactive protein, tumor necrosis factor-α (TNFα), and interferon-γ (IFN-γ) were higher in the rough morphology group of participants [19].

Whether the rough morphology is the direct cause of a more severe MABC infection or if the rough morphology more often affects severely damaged lungs have not yet been established.
One theory is that the smooth variant of MABC initially colonizes abnormal airways and occasionally transforms irreversibly to the rough variant by the loss of glycopeptidolipid expression, causing inflammation and invasive lung disease as a result [14]. In support of this theory, there have been reports of patients followed prospectively where a confirmed transformation from smooth to rough morphology was linked with increased MABC disease severity [23, 24]. The photograph of a mixed colony morphology (Figure 1C) raises the interesting question of whether this is indicative of an infection with 2 different strains or the ongoing transformation from smooth to rough colony morphology of 1 strain. Our study showed increased cavity formation, increased frequency of cough, and poor clinical outcome in study participants with a rough colony morphology isolate.

A general finding of our study, although it focused on the smooth and rough colony morphology, was that we confirm a high validity of the published criteria on how to separate MABC disease from infection or colonization [4]. Of the participants with infection or colonization with MABC rather than disease, none was smear positive and there were no deaths during the study period, despite no treatment. This highlights the importance of considering the criteria for MABC disease to avoid unnecessary treatments.

Our study is limited by its retrospective nature and few study participants, making adjustment for multiple confounders impossible. A further limitation to our study is that only 1 isolate from each patient was cultured. A possible change in morphology during the course of disease and treatment cannot be excluded. However, there is no existing evidence indicating short-term instability of the smooth versus rough colony forming trait in vivo, but in future studies the inclusion of follow-up isolates should be considered. A small study including 28 MABC patients with 71 isolates over time (up to 4 years for some patients), reported stable colony morphology regarding smooth and rough types [13]. Although in our study the determination of colony morphology was as thorough and standardized as possible and no discrepancies between results recorded by the two individuals carrying out this assessment were observed, it was performed visually and interindividual variability cannot be excluded. Furthermore, the data on cavitary lesions on radiograph were retrieved from radiology reports and were not rechecked.

The strengths of our study include the adjustment for age as a potential confounder and inclusion of data on other possible causes of poor treatment outcome, such as subspecies of MABC, inducible macrolide resistance, and weak treatment regimens. Furthermore, the data extracted from medical records are comprehensive as national identity numbers are used in Sweden, enabling data to be retrieved from all health facilities visited.

In conclusion, our study shows that the rough morphology of MABC is associated with poor clinical outcome. Based on our findings and previous supporting data, we suggest that smooth or rough colony forms of MABC should be determined and reported to clinicians due to the potential association with poor clinical outcome. Prospective studies collecting sputum samples regularly during the course of infection is needed to further investigate colony morphology of MABC with regards to transformation and disease course and outcome.

**Supplementary Data**

**Supplementary materials** are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

**Notes**

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